

## ORIGINAL ARTICLE

## KSHV/HHV-8 associated Kaposi's sarcoma in lymph nodes concurrent with Epstein-Barr virus associated Hodgkin lymphoma

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*J Clin Pathol* 2005;58:626–628. doi: 10.1136/jcp.2004.023465

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Accepted for publication  
23 November 2004

**Background:** The unusual occurrence of a metastatic Kaposi's sarcoma (KS) in a lymph node affected by Hodgkin lymphoma (HL) was originally reported when knowledge of the specific virological features of these tumours was lacking.

**Aim:** To re-evaluate this case by assessing whether the simultaneous presence of the two tumours was linked with common aetiopathogenetic factors.

**Methods:** The presence of EBV was investigated by in situ hybridisation, whereas KS associated herpesvirus (KSHV)/human herpesvirus 8 (HHV-8) was detected by immunohistochemistry. Both viruses were analysed in the case reported, in 30 lymph nodes from patients with classic HL, and in 22 skin biopsies from patients with KS.

**Results:** Consistent with the findings in the HL and KS cases analysed, in the case showing features of both HL and KS in the same lymph node, EBV was detectable only in Reed-Sternberg (RS) cells, but not in KS spindle cells, whereas KSHV/HHV-8 was detectable only in KS spindle cells, and not in RS cells.

**Conclusion:** It is probable that the development of KS and HL was related to two independent aetiological cofactors—KSHV/HHV-8 and EBV, respectively—and that the occurrence of the two malignancies in the same patient was merely fortuitous.

About 20 years ago, we described the unusual occurrence of a metastatic Kaposi's sarcoma (KS) in a lymph node affected by Hodgkin lymphoma (HL).<sup>1</sup> The histological diagnosis was based on conventional morphology using haematoxylin and eosin stained tissue sections. Lymph node involvement of both diseases occurred in the manner of a collision tumour. The morphological analysis of the relation between KS and HL revealed some areas of sarcoma merging with areas of lymphoma. Multinucleated giant cells of the Reed Sternberg (RS) type were seen in association with sarcomatous spindle cells, although the histological appearance did not suggest a transition between the two diseases.

"Nowadays, it is possible to use immunohistochemistry and in situ hybridisation to demonstrate unequivocally whether the tumour cells of a specific tumour are infected by either Epstein-Barr virus or Kaposi sarcoma associated herpesvirus"

Epstein-Barr virus (EBV) and Kaposi sarcoma associated herpesvirus (KSHV) are human  $\gamma$  herpesviruses that are aetiological cofactors in the development of a variety of haematological and non-haematological disorders. Both viruses establish persistent latent infection in lymphocytes, which is usually benign. However, in the presence of other environmental, genetic, and iatrogenic cofactors, EBV or KSHV infection is associated with a variety of disorders, including malignant neoplasms.<sup>2–3</sup> Virological and molecular studies have revealed that among the haematological disorders, the development of a small proportion of HLs is associated with infection by EBV,<sup>3</sup> whereas KSHV, also known as human herpesvirus 8 (HHV-8), is consistently associated with KS.<sup>3–4</sup>

Nowadays, it is possible to use immunohistochemistry and in situ hybridisation (ISH) to demonstrate unequivocally

whether the tumour cells of a specific tumour are infected by either EBV or KSHV/HHV-8.<sup>5–6</sup> Therefore, we thought that the case of HD associated with KS in the same lymph node already reported deserved re-evaluation. In fact, we believe that such a re-evaluation could provide additional information and assess whether the simultaneous presence of the two tumours in the same site was a fortuitous event rather than linked with common aetiopathogenetic factors. In particular, we investigated the presence of EBV and KSHV/HHV-8 with the aim of providing additional information on the virological features.

## MATERIALS AND METHODS

The presence of EBV and KSHV/HHV-8 was investigated in the previously reported case displaying the features of both HL and KS in the same lymph node.<sup>1</sup> In addition, lymph node biopsy specimens from 30 patients with classic HL and skin biopsies from 22 patients with KS were examined for comparative purposes. The specimens were fixed in Bouin's solution or in neutral buffered formalin, and then routinely embedded in paraffin wax.

The classification of HL cases was based on immunomorphological features,<sup>7</sup> using anti-CD15 (clone LeuM1; Becton and Dickinson, Oxford, UK), anti CD30 (clone Ber-H2; Dako Cytomation, Glostrup, Denmark), anti-epithelial membrane antigen (anti-EMA; clone E29; Dako Cytomation), anti-latent membrane protein 1 (anti-LMP1; clone CS 1-4; Dako Cytomation), anti-CD20 (clone L26; Dako Cytomation), and anti-CD3 (Dako Cytomation) antibodies.

**Abbreviations:** EBV, Epstein-Barr virus; HHV-8, human herpesvirus 8; HIV, human immunodeficiency virus; HL, Hodgkin lymphoma; ISH, in situ hybridisation; KS, Kaposi's sarcoma; KSHV, Kaposi's sarcoma associated herpesvirus; ORF, open reading fragment; PEL, primary effusion lymphoma; RS, Reed-Sternberg

In all lymph node and skin biopsies, infection by EBV was investigated by ISH, as described previously.<sup>8</sup> The presence of KSHV/HHV-8 was ascertained by immunohistochemistry using a rat monoclonal antibody against the KSHV/HHV-8 latency associated nuclear antigen encoded by viral open reading fragment (ORF) 73 (Advanced Biotechnologies, Columbia, Maryland, USA), as described previously.<sup>9</sup>

The first series of lymph node biopsies with HL came from 19 human immunodeficiency virus (HIV) seronegative patients: 10 patients with mixed cellularity HL and nine with nodular sclerosing HL; age range, 21–82 years; male to female ratio, 14 : 5; seven of these 19 patients were EBV positive. The second series of lymph node biopsies with HL came from 11 HIV infected patients: nine patients with mixed cellularity HL and two with nodular sclerosing HL; age range, 10–45 years; male to female ratio, 7 : 4; 10 of these 11 cases were EBV associated. KS skin biopsies came from 22 HIV infected patients. All the patients with KS were KSHV/HHV-8 positive.

## RESULTS

The KSHV/HHV-8 ORF73 protein was not detectable in RS cells in the HL cases examined, irrespective of the EBV viral status of the RS cells. In addition, EBV infection was consistently absent in the KSHV/HHV-8 positive KS tumour cells.

We also found that in the patient with features of both HL and KS in the same lymph node, the KS component was KSHV/HHV-8 positive and the HL component was EBV positive. Morphologically, the cell populations infected with KSHV/HHV-8 and with EBV were distinct and mainly localised in different areas of the lymph node. One area showed features of KS with vascular proliferation and a population of spindle cells infected by KSHV/HHV-8 (ORF73+, EBV encoded small RNA–), with some of them being close to RS cells and variants (fig 1). Conversely, no KSHV/HHV-8 infected cell was seen to be infected by EBV. Another area showed features of HL, with many binucleated and multinucleated atypical cells and diagnostic RS cells, all infected by EBV (CD15±, CD30+, CD20±, CD3–, EBV

encoded small RNA+, latent membrane protein 1+) (fig 1). No lymphoma cells were infected by KSHV/HHV-8 (ORF73–).

## DISCUSSION

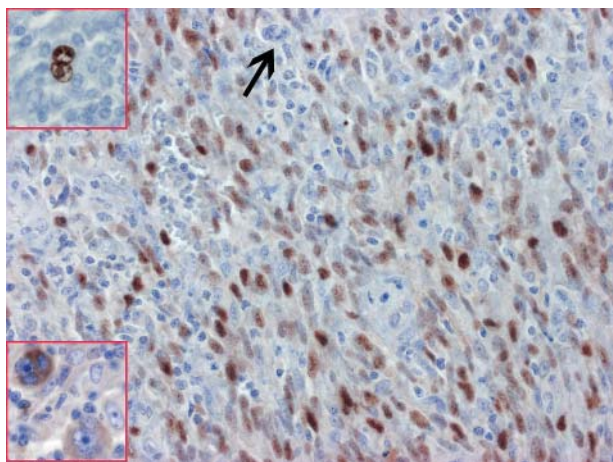
The spectrum of KSHV/HHV-8 associated tumours is different from that associated with EBV. Apart from KS, KSHV/HHV-8 is associated with only a few categories of lymphoproliferative diseases, mostly occurring in patients with HIV infection/AIDS,<sup>3</sup> with primary effusion lymphoma (PEL) being the most frequent.<sup>3</sup> In contrast, evidence accumulated so far strongly supports a role for EBV in the pathogenesis of a wide spectrum of human malignancies. These include not only lymphomas of B, T, and natural killer cell origin and HL, but also lymphoepitheliomas of the nasopharynx, thymus, and stomach, together with leiomyosarcomas arising in organ transplant patients and HIV infected individuals.<sup>2–10</sup>

This study examined KSHV/HHV-8 and EBV viral status in a series of classic cases of HL and KS. As expected, the EBV associated HLs were not infected by KSHV/HHV-8.<sup>11–12</sup> Similarly, the KSHV/HHV-8 associated KS cases were not associated with EBV infection.

Among the human malignancies associated with KSHV/HHV-8 or with EBV, PEL is an interesting exception, because in addition to infection by KSHV/HHV-8, PEL is also often infected by EBV. However, EBV infection tends to associate with AIDS related cases, whereas in immunocompetent hosts PELs are usually EBV negative.<sup>3</sup> At variance with PEL, KS, another KSHV/HHV-8 associated tumour, is not associated with EBV (present study).

*“Evidence accumulated so far strongly supports a role for Epstein-Barr virus in the pathogenesis of a wide spectrum of human malignancies”*

The present study focused on a case which has already been reported<sup>1</sup> because of the morphological coexistence of KS and HL in the same lymph node. It was re-evaluated using immunohistochemistry and ISH techniques for the virological features. This analysis demonstrated the simultaneous occurrence of EBV infected RS cells of HL and of KSHV/HHV-8 infected KS spindle cells. EBV was detected in RS cells only and not in KS spindle tumour cells and, conversely, KSHV/HHV-8 was found only in sarcomatous cells and not in RS cells. Therefore, neither of the tumours was coinfecting by the two viruses. HHV-8 clonality analysis<sup>13</sup> could not be performed in the lymph node with coexistent HD and KS because of the lack of suitable material. In fact,



**Figure 1** Lymph node from a patient presenting with coexisting Kaposi's sarcoma (KS) and Hodgkin lymphoma in the same lymph node. The figure shows that most spindle cells of KS show nuclear staining for the KS associated herpesvirus open reading fragment (ORF) 73 protein. An ORF73 protein negative Reed-Sternberg (RS) cell (arrow) is close to the KS spindle cells. The upper left inset shows an RS cell infected by Epstein-Barr virus (EBV). The lower left inset shows RS cells expressing EBV encoded latent membrane protein 1 (LMP1). Immunohistochemistry for ORF73; upper left (inset), in situ hybridisation for EBV encoded small RNA; lower left (inset) immunohistochemistry for LMP1. Haematoxylin counterstain; original magnification,  $\times 40$ .

## Take home messages

- We re-evaluated a case in which Kaposi's sarcoma (KS) and Hodgkin lymphoma (HL) coexisted in the same lymph node using immunohistochemistry and in situ hybridisation (ISH) for viral analysis
- The HL component was infected with Epstein-Barr virus (EBV) and the KS component was infected with HHV-8/KSHV (human herpesvirus 8/Kaposi's sarcoma associated herpesvirus), but neither tumour was coinfecting by both viruses
- Therefore, KSHV/HHV-8 and EBV were probably independently associated with the development of KS and HL, respectively, and the concurrence of the two malignancies was merely fortuitous
- The virological data confirmed the histopathological diagnosis made at the time of the original report

no frozen tissue had been collected from this retrospectively studied case, and only Bouin's fixed, paraffin wax embedded material was available for special studies.

In conclusion, it is probable that the development of both KS and HL was related to two independent aetiological cofactors—KSHV/HHV-8 and EBV, respectively—and that the concurrence of the two malignancies was merely fortuitous. From a diagnostic point of view, the virological data confirmed the histopathological diagnosis made at the time of the original report. The initial diagnosis was made upon morphological grounds only, mainly because of the lack of knowledge with regard to the specific virological features of these tumours.

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